IX Jornada de Cromatina i Epigenètica

Organitzada per la Secció de Cromatina i Epigenètica de la Societat Catalana de Biologia (SCB)

amb el Barcelona Chromatin Club (BCC)

IX Annual Chromatin and Epigenetics symposium

Organized by the Chromatin and Epigenetics section of the Catalan Society of Biology (SCB)
—Albert Jordan—

and the Barcelona Chromatin Club (BCC)
—Sonia Forcales—

March 22, 2019

IEC, carrer del Carme, 47, Barcelona
Prat de La Riba hall

Sponsored by:

PROGRAM

***8.20-8.50** Registration and documentation pickup*
***8:50** Opening*

Session I. *Chair:* ***Sandra Peiró***

9:00-9:20 **15min +5**
Manel Esteller (IJC, PEBC-IDIBELL)
Epigenetic reprogramming of the cancer cell proteome

9:20-9:40 **15min +5**
Sonia Forcales (UB-IDIBELL, PMPPC-IGTP)
Binding of RNA processing proteins to a novel macrosatellite-derived lncRNA in colorectal cancer

9:40-10:00 **15min +5**
Jorge Ferrer (CRG, CIBERDEM, Imperial College London)
Non-coding genome function and diabetes

10:00-10:20 **15min +5**
Sara Sánchez (Fund. Sant Joan de Déu)
Chromatin remodeling in Ewing Sarcoma

10:20 Short Talk 1 **7min +3**
Laura Barba (CRG)
Dynamics of DYRK1A-dependent BRCA1 chromatin occupancy

10:30-10:35 Sponsor' talk **5min**
Marcos Arranz (Genes-MDPI)

***10:35-11:15** Coffee break and poster session*

11:15-11:20 Sponsor' talk **5min**
Sarantis Chlamydas (Active Motif)
Focus on chromatin

Session II. –BCC9. 3D nuclear organization
Chair: ***Marc Martí-Renom***

11:20-11:50 **25min +5**
Olivier Cuvier (CNRS-Univ. Toulouse)
3D epigenetic heterochromatin spreading and chromatin-encoded cell-to-cell variability

11:50-12:20 **25min +5**
José Carlos Reyes (CABIMER, Sevilla)
Coexpression, corregulation and 3D nuclear organization

12:20 Short Talk 2 **7min +3**
Sergi Aranda (CRG)
Chromatin capture links the metabolic enzyme AHCY to stem cell proliferation

12:30 Short Talk 3 **7min +3**
Noelia Díaz (Max Planck Inst. Mol. Biomed.)
Chromatin conformation analysis of primary patient tissue using a low input Hi-C method

12:40 Short Talk 4 **7min +3**
Irene Farabella (CNAG-CRG)
An Integrative 3D Modelling Method for Chromosome Structure Determination Using Super-Resolution Imaging and Hi-C

12:50 Short Talk 5 **7min +3**
Marco di Stefano (CNAG-CRG)
Bridging the epigenetic features and the genome 3D organization in Arabidopsis thaliana

13:00 Short Talk 6 **7min +3**
Rene Bottcher (IRB)
Rapid reversible changes in compartments and local chromatin organization revealed by hyperosmotic shock

13:10 Short Talk 7 **7min +3**
Renee Beekman (IDIBAPS)
Integration of genomic and epigenomic data, including the 3-dimensional chromatin structure, refines the link between genetic predisposition and the gene regulatory landscape at chronic lymphocytic leukemia risk loci

13:20-14:30 ***Lunch and poster session***

Session III. *Chair:* ***Àlex Vaquero***

14:30-14:50 **15min +5**
Ferran Azorin (IBMB-CSIC, IRB)
Centromeric chromatin and the co-ordination of mitosis

14:50-15:10 **15min +5**
Sara Pagans (IDIBGI-Univ. Girona)
Regulome-seq analysis of genetic variation at cis-regulatory regions of human cardiac genes

15:10-15:30 **15min +5**
Rafael Oliva (IDIBAPS, UB)
Contribution of the sperm chromatin to the development and epigenome of the embryo

15:30-15:50 **15min +5**
Guillermo Vicent (CRG)
C/EBPa crosstalks with progesterone receptor to control hormone-dependent cell growth in breast cancer cells

15:50 Short Talk 8 **7min +3**
Mariona Nadal (IRB)
Sensitive high-throughput single-cell RNA-Seq reveals within-clonal transcript-correlations in yeast populations

16:00 Short Talk 9 **7min +3**
Elena Vizcaya (UB)
The regulatory genome of Drosophila regeneration

16:10 Short Talk 10 **7min +3**
Beatriz Pérez (IGTP)
Epigenetic regulation of the Kallikrein family

16:20-16:50 ***Coffee break and poster session***

Session IV. *Chair:* ***Sonia Forcales***

16:50-17:10 **15min +5**
Albert Jordan (IBMB-CSIC)
Human histone H1 variants: distribution and especificities

17:10-17:30 **15min +5**
Sandra Peiró (VHIO)
Chromatin dynamics in EMT

17:30-17:50 **15min +5**
Josep Jimenez (Fund. Sant Joan de Déu)
Childhood obesity: Mixing snowballs with epigenetics

17:50 Short Talk 11 **7min +3**
Lukas Ded (Charles Univ., Prague)
Estrogen alters histone-to-protamine transition process and epigenetic profiles in testis and sperm

18:00 Short Talk 12 **7min +3**
Sergi Cuartero (CRG, Imperial College London)
Cohesin links inducible gene expression and myeloid differentiation

18:10 Short Talk 13 **7min +3**
Marina Ruiz (CRG)
FLEA-ChIP enables reproducible immunoprecipitation from 100 cells

18:30 ***Meet together for a Beer***

POSTERS

1. Anna Ardèvol (URV)
Long-lasting effects of gspe on ileal glp-1 gene expression are associated to a hypomethylation of the glp-1 promoter

2. Alba Azagra (PEBC-IDIBELL)
Unveiling a novel function of the transcriptional repressor HDAC7 during early B cell development

3. Celia Corral (UAB)
Biomarker pairs of sperm miRNA: new tools for a molecular fertility evaluation

4. Oriol de Barrios (IDIBELL)
HDAC7, a novel biomarker and potential therapeutic target for infant pro-B-ALL with MLL-AF4 rearrangement

5. Antoni Gañez (Stockholm Univ.)
SWI/SNF subunits Brg1 and Brm affect alternative splicing by regulating the recruitment of splicing associated factors

6. Erica Hurtado (IGTP)
HDAC11 deficiency induces a shift in skeletal muscle fiber type

7. Simona Iacobucci (IBMB-CSIC)
Impact of PHF8 histone demethylase in astrocytes differentiation

8. Vanesa Izquierdo (UB)
Differential microRNAs expression profile in the hippocampus of senescent mice offspring induced by maternal resveratrol supplementation

9. Paula Jauregui (UB)
H4K5 post-translational modifications dynamics on sperm chromatin remodeling

10. Carlos Jimenez (VHIR)
Targeting the oncogenic role of the chromatin remodeler BRG1 in Neuroblastoma

11. Julen Mendieta (CNAG-CRG)
3D genome structure reconstruction from sparse 3C-based datasets

12. Francesca Mugianesi (CNAG-CRG)
Dynamic regulation of chromatin through 3D epigenetic clusters

13. Clara Penas (UAB)
BET proteins as a novel target to reduce inflammation and enhance functional recovery after SCI

14. Laia Ribas (ICM-CSIC)
Identification of gonadal miRNAs in zebrafish exposed to high temperature during early stages of development

15. Marta Vicioso (IBMB-CSIC)
JMJD3-mediated chromatin re-organization controls transcription upon TGFb signaling

16. Laura García (CRG)
Does chromatin metabolism drive breast cancer evolution?

17. Eudald Pascual (UB)
Cis-Regulatory Element (CRE) dynamics during planarian posterior regeneration

18. Joan Gil (IGTP)
The DNA methylation of the Kallikrein locus as a pancancer biomarker

19. Ada Soler (IDIBAPS)
Characterization of human sperm protamine proteoforms through a combination of top-down and bottom-up mass spectrometry approaches

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ABSTRACTS

Short Talk 1

Dynamics of DYRK1A-dependent BRCA1 chromatin occupancy

Laura Barba[1,2], Chiara Di Vona[1,2], Rianne Cort[1,2] and Susana de la Luna[1,2,3]

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3- Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

DYRK1A (dual-specificity tyrosine-regulated kinase 1A) belongs to a conserved family of protein kinases -DYRK- present in all eukaryotes. DYRK1A is known to participate in cell proliferation and differentiation decisions and to fulfill key roles in brain development. Moreover, dysregulation of DYRK1A has pathological consequences in humans. We have shown that DYRK1A binds to proximal promoter regions enriched in a conserved palindromic motif and acts as a transcriptional activator of target genes. To uncover potential additional factors participating in this process, we analyzed ChIP-Seq data from ENCODE and found that the tumor suppressor BRCA1 is recruited to the DYRK1A-consensus motif. Our results indicate that BRCA1 is in nuclear complexes with the kinase and that it is a substrate of DYRK1A. Indeed, inhibition of DYRK1A kinase activity by the specific inhibitor harmine results in a strong decrease of BRCA1 recruitment to chromatin, supporting a role for DYRK1A in BRCA1 recruitment to its genomic *loci*. Given the fact that both proteins play roles in the DNA damage response, we have explored the putative interplay between DYRK1A and BRCA1 at chromatin in response to DNA damage. Upon induction of DNA double strand breaks with etoposide, BRCA1 mobilizes to novel genomic loci, mostly promoter regions enriched in the DYRK1A-associated consensus motif. The DNA damage-induced BRCA1 *loci* are co-occupied by DYRK1A, and correspond mostly to actively transcribed genes, as indicated by the presence of elongating RNA polymerase II. Interestingly, harmine treatment leads to a significant reduction of BRCA1 chromatin occupancy at those BRCA1 gained-*loci*, suggesting that DYRK1A-dependent phosphorylation of BRCA1 is required for its mobilization to target *loci* in response to DNA damage.

Short Talk 2

Chromatin capture links the metabolic enzyme AHCY to stem cell proliferation

Sergi Aranda*, Anna Alcaine-Colet, Enrique Blanco, Eva Borràs, Claire Caillot, Eduard Sabidó, Luciano Di Croce*

Profiling the chromatin-bound proteome (chromatome) in a simple, direct, and reliable manner might be key to uncovering the role of yet uncharacterized chromatin factors in physiology and disease. Here, we have design an experimental strategy to survey the chromatome of proliferating cells by using the DNA-mediated chromatin pull-down technology (Dm-ChP). Our approach provides a global view of cellular chromatome in normal physiological conditions and enables the identification of chromatin-bound proteins *de novo*. Integrating Dm-ChP with genomic and functional data, we have discovered an unexpected chromatin function for adenosylhomocysteinase (AHCY), a major one-carbon pathway metabolic enzyme, in gene activation. Our study reveals a new regulatory axis between the metabolic state of pluripotent cells, ribosomal protein production, and cell division during early phase of embryo development, in which the metabolic flux of methylation reactions is favored in a local milieu.

Short Talk 3

Chromatin Conformation Analysis of Primary Patient Tissue Using a Low Input Hi-C Method

Noelia Díaz[1,6], Kai Kruse[1,6], Tabea Erdmann[2], Annette M. Staiger[3,4,5], German Ott[3], Georg Lenz[2], and Juan M. Vaquerizas[1]

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Chromatin conformation constitutes a fundamental level of eukaryotic genome regulation. However, our ability to examine its biological function and role in disease is limited by the large amounts of starting material required to perform current experimental approaches. Here, we present Low-C, a Hi-C method for low amounts of input material. By systematically comparing Hi-C libraries made with decreasing amounts of starting material we show that Low-C is highly reproducible and robust to experimental noise. To demonstrate the suitability of Low-C to analyse rare cell populations, we produce Low-C maps from primary B-cells of a diffuse large B-cell lymphoma patient. We detect a common reciprocal translocation t(3;14)(q27;q32) affecting the *BCL6* and IGH loci and abundant local structural variation between the patient and healthy B-cells. The ability to study chromatin conformation in primary tissue will be fundamental to fully understand the molecular pathogenesis of diseases and to eventually guide personalised therapeutic strategies.

Short Talk 4

An Integrative 3D Modelling Method for Chromosome Structure Determination Using Super-Resolution Imaging and Hi-C.

Irene Farabella[1]*, Guy Nir[2]*, Cynthia Perez Estrada[3]*, Carl Ebeling[6]*,Brian J. Beliveau[2,4,5], Hiroshi M. Sasaki[4,5], Soun H. Lee[2], Son C. Nguyen[2†], Ruth McCole[2], Shyamtanu Chattoraj[2], Jelena Erceg[2], Jumana AlHaj Abed[2], Nuno M. Martins[2], Huy Nguyen[2], Mohammed Hannan[2], Shiekh Russell[3], Steven Callahan[7], John Schreiner[7], Jeff Stuckey[6]*, Peng Yin[4,5]*, Erez Lieberman Aiden[3,9,10,11]*, C.-ting Wu[2,4]*, Marc Martí Renom[1,12,13]*.

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Deciphering the principles of chromatin spatial arrangement in the nucleus is essential to understand chromatin functions and how it affects gene expression. However, characterizing this highly complex three-dimensional (3D) organization remains a challenge. Advances in DNA fluorescent *in situ* hybridization imaging (FISH) (such as the use of Oligopaint probes in combination with STORM and DNA-PAINT) and Chromosome Conformation Capture (3C) based methods allowed to shed light on the chromatin kilobases to megabases scale folding principles, unveiling a complex multi-scale organisation of structural domains. Modelling the spatial arrangement of such structural domains with respect to each other in cell population has been so far elucidated using data driven or polymer physics modelling approaches. These are viable approaches that shed light on averaging conformations of many chromosomes and helped to infer important biological insight driven by the genomic 3D organisation. How these compartments are packed in 3D at single cell level is only now starting to emerge. To model chromatin spatial arrangement in the contest of a single diploid nucleus we developed a novel integrative modelling approach that combines single cell imaging experiments (as OligoSTORM) with cell ensemble 3C experiments (as Hi-C). We applied our method on 8.16 Mb of Chr19 in human PGP1 fibroblast cell line and successfully decipher for the first time the 3D conformation of two homolog regions of Chr19 in a diploid nucleus, unveiling their folding differences and similarities.

Short Talk 5

Bridging the epigenetic features and the genome 3D organization in *Arabidopsis thaliana*

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The *Arabidopsis thaliana* genome has been widely characterized in its spatial and epigenetic organizations. Microscopy revealed, for example, that the nucleolus is constituted by specific sequences (nucleolar organizing regions, NORs), the centromeres are located at the nuclear periphery, and the telomeres are recruited around the nucleolus. Additionally, the chromosome conformation capture (Hi-C) interaction map shows typical patterns such as stripes and long-range loops. Finally, the extensive epigenetic analysis revealed the partition of the genome in at least four states: NORs, constitutive and facultative heterochromatin, and active chromatin. However, a comprehensive interpretation of all these experimental findings is still missing. Here, we used polymer chromosome models and molecular dynamics to test whether the large-scale features of the *A. thaliana* genome can be explained by using simple short-range interactions (attraction or repulsion) between chromosome regions of different epigenetic states. In our approach, chromosomes are represented as hetero-polymers in which each 3kilo-bases monomer is assigned to one of the four epigenetic states. We found that the attraction between NORs particles induces the formation of the nucleolus, that the repulsion between heterochromatic regions and the other states favors both the formation and the preferential location at the nuclear periphery of the centromeres, and the attraction between active particles allows to recapitulate many of the contact patterns revealed in the Hi-C map. Finally, by considering which aspects are poorly reconstructed by our epigenetic-based study, we highlight other possible mechanisms such as long range looping that could be used in future studies.

Short Talk 6

Rapid reversible changes in compartments and local chromatin organization revealed by hyperosmotic shock.

Amat R[#1,2], Böttcher R[#1,2], Le Dily F[#3], Vidal E[3], Quilez J[3], Cuartero Y[3,4], Beato M[3,4,5], de Nadal E[1,2], Posas F[1,2].

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Abstract
Nuclear architecture is decisive for the assembly of transcriptional responses. However, how chromosome organization is dynamically modulated to permit rapid and transient transcriptional changes in response to environmental challenges remains unclear. Here we show that hyperosmotic stress disrupts different levels of chromosome organization, ranging from A/B compartment changes to reduction in the number and insulation of topologically associating domains (TADs). Concomitantly, transcription is greatly affected, TAD borders weaken, and RNA Polymerase II runs off from hundreds of transcription end sites. Stress alters the binding profiles of architectural proteins, which explains the disappearance of local chromatin organization. These processes are dynamic, and cells rapidly reconstitute their default chromatin conformation after stress removal, uncovering an intrinsic organization. Transcription is not required for local chromatin reorganization, while compartment recovery is partially transcription-dependent. Thus, nuclear organization in mammalian cells can be rapidly modulated by environmental changes in a reversible manner.

Short Talk 7

Integration of genomic and epigenomic data, including the 3-dimensional chromatin structure, refines the link between genetic predisposition and the gene regulatory landscape at chronic lymphocytic leukemia risk loci

Beekman Renée[1,2*], Speedy Helen E.[3*], Chapaprieta Vicente[4], Orlando Giulia[3], Law Philip J.[3], Martín-García David[1,2], Gutiérrez-Abril Jesús[5], Catovsky Daniel[3], Beà Sílvia[1,2], Guillem Clot[1,2], Montserrat Puiggros[6], David Torrents[6,7], Puente Xose S.[2,5], Allan James M.[8], López-Otín Carlos[2,5], Campo Elias[1,2,4,9], Houlston Richard S.[3**], Martín-Subero José I.[1,2,4,7**]

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Genome-wide association studies have provided evidence for inherited predisposition to chronic lymphocytic leukemia (CLL), identifying 42 (non-HLA) genomic regions influencing CLL risk. However, efforts defining mechanisms mediating the risk at these, largely non-coding, loci have been constrained by a lack of integrated genome-wide data in large CLL series.

Here we aimed to refine the gene regulatory mechanisms and biological significance of CLL risk loci by (i) analysing high-resolution chromatin state maps and integrating genetic, epigenetic and transcriptomic information in primary CLL cases, (ii) performing *in silico* transcription factor (TF) binding analysis and (iii) studying the three-dimensional (3D) chromatin structure using promoter capture Hi-C in normal B cells and CLL.

Eighty-one percent of the risk loci were enriched for regulatory elements in CLL, suggesting a regulatory role for these loci in CLL pathogenesis. More specifically, 30 risk loci were associated with genome activity (H3K27ac), chromatin accessibility (ATAC-seq) and/or gene expression quantitative trait loci (QTLs) in up to 452 primary CLLs. Yet, we characterised the gene regulatory mechanisms at these loci in closer detail by showing that genotypes associated with higher CLL risk result in decreased affinity for B-cell related TFs and increased affinity for FOX, NFAT and TCF/LEF TF family members. Lastly, we observed significant 3D chromatin interactions in CLL and normal B cells between the risk loci and 15 eQTL gene loci, suggesting direct regulatory links between risk loci and target gene expression in relation to CLL predisposition. Importantly, CLL risk loci not necessarily targeted their nearest genes but also influenced expression of distant genes via long-range chromatin interactions.

By characterising regulatory elements and TFs playing a role in mediating the effects of genetic variation at CLL risk loci and determining downstream genotype-dependent effects on gene expression, we offer improved insights into the functional and biological basis of CLL predisposition.

Short Talk 8

Sensitive high-throughput single-cell RNA-Seq reveals within-clonal transcript-correlations in yeast populations

Mariona Nadal-Ribelles[1,2,3,4†], Saiful Islam[1,2†], Wu Wei[1,2,5†], Pablo Latorre[3,4†], Michelle Nguyen[1,2], Eulàlia de Nadal[3,4], Francesc Posas[3,4], Lars M. Steinmetz[1,2,6*].

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Abstract

Single-cell RNA-seq has revealed extensive cellular heterogeneity within many organisms, but few methods have been developed for microbial clonal populations. The yeast genome displays unusually dense transcript spacing, with interleaved and overlapping transcription from both strands, resulting in a minuscule but complex pool of RNA protected by a resilient cell wall. Here, we have developed a sensitive, scalable, and inexpensive yeast single-cell RNA-seq (yscRNA-seq) method that digitally counts transcript start sites in a strand- and isoform-specific manner. YscRNA-seq detects the expression of low-abundant, non-coding RNAs, and at least half of the protein-coding genome in each cell. Within clonal cells, we observed a negative correlation for the expression of sense/antisense pairs, while paralogs and divergent transcripts co-express. Combining yscRNA-seq with index sorting, we uncovered a linear relationship between cell size and RNA content. Although we detected an average of ~3.5 molecules/gene, the number of expressed isoforms are restricted at the single-cell level. Remarkably, the expression of metabolic genes is highly variable, while their stochastic expression primes cells for increased fitness towards the corresponding environmental challenge. These findings suggest that functional transcript diversity acts as a mechanism for providing a selective advantage to individual cells within otherwise transcriptionally heterogeneous populations.

Short Talk 9

THE REGULATORY GENOME OF *DROSOPHILA* REGENERATION

Elena Vizcaya-Molina[1], Cecilia C. Klein[2], Florenci Serras[1], Roderic Guigó[2] and Montserrat Corominas[1]

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One of the most important questions in regenerative biology is how and when genes change expression to trigger regeneration programs. Resetting of gene expression patterns during injury responses is shaped by the coordinated action of genomic regions that integrate the activity of multiple sequence-specific DNA binding proteins. Using genome-wide approaches to interrogate chromatin function we identify the regulatory elements governing tissue recovery in *Drosophila* imaginal discs, which show a high regenerative capacity after genetically induced cell death. Our findings indicate a global co-regulation of gene expression as well as the existence of a regeneration program driven by different types of regulatory elements. Novel enhancers acting exclusively in the damaged tissue cooperate with enhancers co-opted from other tissues and developmental stages, and with endogenous enhancers that show increased activity after injury. These enhancers host binding sites for regulatory proteins, including a core set of conserved transcription factors that regulate regeneration across metazoans.

Short Talk 10

Epigenetic regulation of the Kallikrein family

Beatriz Pérez, Joan Gil, Núria Villalmanzo, Mireia Jordà

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Kallikreins (KLKs) are a family of 15 serine proteases implicated in a vast range of physiological processes (skin desquamation, synaptic plasticity, etc.). They have been associated with different pathologies, mainly with cancer disease, such as KLK3 (PSA) as a biomarker of prostate cancer. This family represents one of the biggest genetic cluster in the human genome (around 265kb) but the regulation at a genomic level is still poorly understood. Previous results from our laboratory showed that some KLKs display aberrant DNA methylation in thyroid cancer. Moreover, the analysis of their expression revealed differential and specific expression profiles associated with the main driver mutations in thyroid cancer, *BRAF* or *RAS*, outlining three different domains of expression (A, B and C). Importantly, these domains were maintained in other types of cancer, although displaying different profiles. Therefore, we hypothesize that the KLK cluster is divided in three expression domains that are regionally coregulated through cis interactions between KLKs promoters from different domains and from the same domain and, additionally, distal regulatory elements. First, we identified a region located between domain A and B that is predicted by chromatin state available data from ENCODE to be a strong enhancer only in those cell lines that express the domains B and C of the KLK cluster. Performing luciferase reporter assays, we confirmed its enhancer activity, which was higher in cells expressing domains B and C than in cells not expressing KLKs. Preliminary results checking the DNA methylation levels of the enhancer seem to point out that this epigenetic mechanism is playing a minor role in the regulation of this cluster. Further analysis are currently being performed, such as UMI-4C and CRISPR, to better understand the underlying mechanism of regulation of the KLK family.

Short Talk 11

ESTROGEN ALTERS HISTONE-TO-PROTAMINE TRANSITION PROCESS AND EPIGENETIC PROFILES IN TESTIS AND SPERM

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Estrogens are a group of steroid compounds, named for their importance in the estrous cycle. They have been for a long time considered mainly as female hormones, but they also play an important role in regulating male reproductive functions. In our previous studies we described the significant effect of several types of natural and synthetic estrogens on various aspects of sperm and testicular physiology. Despite the large body of evidence about the effect of estrogens on male reproductive functions, the epigenetic effect of estrogens on the process of spermatogenesis and its physiological consequences remains largely unexplored. We carried out transgenerational *in vivo* study on mice to study the effect of synthetic estrogen 17α-Ethynylestradiol (EE2) in two doses (environmental - 2.5 ng/L and anticonception - 2.5 µg/L) on the process of histone-to-protamine transition in testicular tissue, epigenetic profile in testicular cells/sperm in males of P generation and to evaluate the effect of EE2 on subsequent F1, F2 generations (with and without continuous exposure). We observed significantly higher retention of histones in mature sperm nuclei in the group of P males exposed to higher dose of EE2 (55.4 ± 9.7 RFU vs 69.1 ± 11.2 RFU). The effect was also propagated to F1 generation, but not observable in F2 generation. The changes in histones abundancies were also accompanied by the changes in their post-translational modifications (H4K12Ac, H3K27me3, H3K36me2) with the prominent effect on H4K12Ac (66.4 ± 7.9 RFU vs 49.2 ± 9.5 RFU). Our ongoing work is now focused on the evaluation of the testicular tissue sections and individual testicular cell types to determine the exact points of the effect of EE2 on epigenetic profiles during spermatogenesis. Our further work on the presented project should bring new important insights into the process of the hormonal regulation of epigenetic functions in males.

This work was supported by the project "BIOCEV" – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University" (CZ.1.05/1.1.00/02.0109), from the European Regional Development Fund (www.biocev.eu), by the project International Mobility of Researchers of Biotechnologický ústav AV ČR, v. v. i. (CZ.02.2.69/0.0/0.0/16_027/0008353) from the ERDF, by the Grant Agency of the Czech Republic No. GA-18-11275S, by the Charles University in Prague No. SVV260440, by the Institutional support of the Institute of Biotechnology RVO: 86652036

Short Talk 12

Sergi Cuartero

CRG, Imperial College London

Cohesin links inducible gene expression and myeloid differentiation

Cohesin is important for 3D genome organisation in interphase. Nevertheless, even the complete removal of cohesin has surprisingly little impact on steady-state gene transcription and enhancer activity. Here we show that cohesin is critical for the core transcriptional response of primary macrophages to microbial signals, and for inducible enhancer activity that underpins inflammatory gene expression. Consistent with the role of inflammatory signals in promoting myeloid differentiation of haematopoietic stem and progenitor cells (HPSC), cohesin mutations in HSPC led to reduced inflammatory gene expression, and increased resistance to differentiation-inducing inflammatory stimuli. These findings uncover an unexpected dependence of inducible gene expression on cohesin, link cohesin with myeloid differentiation, and help explain the prevalence of cohesin mutations in human acute myeloid leukaemia.

Short Talk 13

FLEA-ChIP enables reproducible immunoprecipitation from 100 cells

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Chromatin immunoprecipitation (ChIP) is the preferred technique to uncover *in-vivo* interactions between proteins and DNA regions. The emergence of the Next-Generation Sequencing technologies and their application to the detection of the immunoprecipitated DNA has allowed for a dramatic increase of the throughput, the resolution and the sensitivity of the ChIPs. The increase in the sensitivity of detection has prompted researchers to challenge the input material to perform the assay. The identification of cell-specific interactions will allow for untangling the epigenetic differences existing within cell populations, as well as to uncover co-occurrence of chromatin modifications. Although some approaches have been reported in this direction, the low amount of immunoprecipitated DNA still represents a challenge when performing libraries for next-generation sequencing.

To fill this technological gap we implemented a fast, low-input, easy and affordable ChIP (FLEA-ChIP). This assay is an evolution of the previously reported iChIP (indexed-first) assay. In the latter, chromatin from ultra-low-input samples is immobilized in proteinA-magnetic beads and indexed prior to immunoprecipitation. However, we performed several improvements to support further reduction of the input material and a deeper-sequencing of immunoprecipitated DNA, allowing for ChIP-Seq sample generation from up to 100 cells for a fair cost (140$ including sequencing). Our approach offers advantages to the currently available protocols: (i) It is highly reproducible; (ii) it can work with frozen samples; (iii) it does not require specific and sophisticated machinery; and (iv) it permits, both, pooling of several samples to reduce technical variability, as well as performing individual assays for those cases in which the sample amount is an important constrain. We applied FLEA-ChIP to produce ChIP-Seq data for the study of chromatin dynamics of *Drosophila* imaginal tissues during differentiation using a wide collection of antibodies. Our results confirm that FLEA-ChIP is an excellent and versatile approach when working with very-low input samples, and a more reliable and reproducible assay to find out differences between several conditions.

In conclusion, FLEA-ChIP protocol is an adaptable method to any low-input condition as low as 100 human cells. It is affordable for all laboratories thanks to a non-sophisticated procedure and its economic budget. Its implementation will favor the production of high quality, replicable, trustworthy results and it allows chromatin immunoprecipitation assays to get a bit closer to the single-cell era.

Sponsor talk

Sarantis Chlamydas

Active Motif

TECHNICAL TALK: NOVEL TOOLS IN EPIGENETIC RESEARCH

Epigenetics, involves an additional layer of information on top of the nucleotide sequence without changing it. This complexed information is mainly based on chemical modifications. These modifications include DNA and RNA methylation as well as post translation modifications of chromatin structure proteins called histones. The study of these reversible changes, elucidates mechanisms of action of DNA binding transcription factors, protein-protein interactions, mediating changes in gene expression and transcriptome profiling in cases of human disorders, in different cell types and tissues. Recent studies in the area of neurobiology, cancer and metabolism have revealed the importance of epigenetic regulation in specific gene pathways and cascades.

It is clear that a new era of epigenetics study is now possible, thanks to modern high-throughput techniques like Reduced Representation Bisulfite sequencing (RRBS), ChIP-seq in clinical samples(Biopsies, FFPE), ChIP-seq followed by Mass Spec, Mass Spec analysis of Histone Modifications (Mod Spec) and ATAC seq. These new techniques are mainly dependent upon high quality, proven reagents and high scientific expertise, suggesting that research reagents like specific, validated antibodies and recombinant enzymes for RNA, DNA and histone modifications, are still as relevant and necessary in this field today as they have ever been.

In this technical talk we will highlight our recent advances in the field of chromatin biology and gene regulation. We will present an overview of our novel tools, epigenetic reagents and discuss a series of data generated by our RnD and Epigenetics Services laboratories in the area of drug discovery and human diseases.

Poster 1

LONG-LASTING EFFECTS OF GSPE ON ILEAL GLP-1 GENE EXPRESSION ARE ASSOCIATED TO A HYPOMETHYLATION OF THE GLP-1 PROMOTER

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A grape seed proanthocyanidin extract (GSPE) presents long-lasting effects, reducing body weight gain and increasing lipid oxidation in cafeteria-diet-fed animals. It has also shown to modulate the enteroendocrine system. In this paper we determine the role of several GSPE treatments, previously shown to alter body weight gain and/or respiratory quotient in cafeteria-diet-fed rats, in the gene expression of several enterohormones and to ascertain whether they are modulated by epigenetic mechanisms. We found that 10-day GSPE administration prior to administration of the cafeteria diet (pre-treatment) led to upregulation of GLP-1 mRNA in the ileum 17 weeks after the GSPE treatment that was associated with hypomethylation on the GLP-1 promoter. These effects were also found when GSPE treatment was maintained as simultaneous-intermittent treatment (administered every other week) during the 17 weeks of cafeteria-diet treatment. Also, the hypomethylation of the GLP-1 promoter correlated positively with body weight, respiratory quotient and plasma insulin. In the colon, GSPE had no effect on gene expression after pre-treatment. On the other hand, GSPE administered at the end of the cafeteria diet upregulated PYY and GLP-1 mRNA, though it was not regulated by either the hypomethylation of the promoters or the acetylation of H3K9 and tri-methylation of H3K27 histones on the GLP-1 promoter. In conclusion, we have identified long-lasting effects of GSPE on GLP-1 gene expression in the ileum. These were partly mediated by a reduction in methylation at the gene promoter, which in turn was associated with changes in body weight, energy expenditure and plasma insulin.

Poster 2

Unveiling a novel function of the transcriptional repressor HDAC7 during early B cell development

Alba Azagra[1], Olga Collazo[1], Maria Vila-Casadesús[2], Thomas Graf[2], Maribel Parra[1]

B lymphopoiesis is the result of several cell lineage choices and differentiation steps whose perturbation leads to B cell malignancies. Cellular transitions for B cell generation have been associated with gene activation by networks of B cell specific transcription factors (TFs) and dynamic changes in DNA methylation. Evidence, however, supports also critical gene silencing functions of these TFs. How gene repression is established and which lineage-specific transcriptional repressors are involved during B cell lymphopoiesis are still not totally understood. Our group has previously reported that the transcriptional repressor HDAC7 is highly expressed in B cell progenitors (pro-B cells) and B cell precursors (pre-B cells) but not in myeloid cells such as macrophages. Using an *in vitro* system (reprogramming of pre-B cells into macrophages) and an *in vivo* experimental approach (mouse model for specific deletion of HDAC7 in pro-B cells), we have demonstrated that HDAC7 is essential for early B cell development and the acquisition of proper B cell identity. We found that HDAC7 represses myeloid and T lymphocyte genes in pro-B cells through interaction with the TF MEF2C. In normal pro-B and pre-B cells HDAC7 is recruited to the promoters and enhancers of lineage inappropriate genes, leading to their transcriptional silencing. Here, by using our *in vivo* experimental approach, we have found that HDAC7 represses *Tet2* (an enzyme that converts 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) resulting in DNA demethylation and directing gene activation) in pro-B cells. Microarray and RT-qPCR analysis show that *Tet2* expression is up-regulated in HDAC7 deficient pro-B cells. Graf and colleagues previously demonstrated that *Tet2* is up-regulated during the transdifferentiation of pre-B cells into macrophages. Conversely, we have found that HDAC7 is down-regulated during the conversion of pre-B cells into macrophages and that its exogenous expression blocks the up-regulation of Tet2. Chromatin immunoprecipitation (ChIP) experiments revealed that HDAC7 is recruited to the promoter and enhancer of the *Tet2* gene in pro-B cells and its absence leads to an increase and a decrease in active and repressive histone marks, respectively. Additionally, we have found that the absence of HDAC7 from pro-B cells results in a significant increase in the percentage of global 5-hydroxymethylation. To definitively prove the role of HDAC7 in 5-hydroxymethylation, we have performed a genome-wide experimental approach. hMeDIP-sequencing experiments reveal an increase in the enrichment of this epigenetic modification at many loci related to lineage inappropriate genes. Finally, we have observed 5-hmC enrichment at retrotrasposon elements (LINE-1) in HDAC7 deficient pro-B cells, suggesting a potential protector function of HDAC7 against chromatin instability and DNA damage. Altogether, our results indicate that HDAC7 is an essential polyvalent transcriptional regulator during early B cell development that silences lineage or functionally inappropriate genes and unveils an unexpected role of a class IIa HDAC in controlling DNA methylation.

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Poster 3

Biomarker pairs of sperm miRNA: new tools for a molecular fertility evaluation

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Male infertility is commonly assessed trough seminogram analysis. Nevertheless, the limitations of this assay have encouraged the search for trustworthy molecular biomarkers. Research in this area has revealed that certain pairs of sperm transcripts display a correlative expression in fertile populations which is altered in infertile individuals. In this context, this study has been aimed at identifying pairs of sperm miRNAs that display a good potential role as fertility biomarkers.

With this purpose, semen samples were collected from a fertile group of individuals (n=10) and four infertile populations with different seminal characteristics: asthenozoospermia (A; n=10), teratozoospermia (T; n=10), oligozoospermia (O; n=10), and normozoospermia (unexplained male infertility; UMI; n=8). Sperm RNA from each individual was isolated and the expression pattern of 736 miRNAs was characterized by qRT-PCR. Correlation between expression values of every possible miRNA-miRNA combination was analyzed (Spearman). Among the 48 miRNA pairs that showed a stable significant correlation in fertile males, the ubiquitous pairs with a non-correlated expression in each infertile population were selected. The relative expression of these pairs was calculated ($expression_{miRNA1}$ – $expression_{miRNA2}$) and evaluated by Receiver Operating Characteristic curve analyses in order to determine their biomarker potential. For each infertile population, the pair that obtained the highest Area Under the Curve (AUC) was selected: A: hsa-miR-942-5p/hsa-miR-1208 (AUC=0.91); T: hsa-miR-296-5p/hsa-miR-328-3p (AUC=0.87); O: hsa-miR-139-5p/hsa-miR-1260a (AUC=1); and UMI: hsa-miR-34b-3p/hsa-miR-93-3p (AUC=1). The relative expression of these pairs was verified to be significantly differential between fertile and infertile individuals (Mann-Whitney; $p<0.05$). The selected miRNA pairs display a remarkable potential as a male infertility biomarker panel that could serve as a molecular complement of seminogram analyses, especially when facing cases of uncertain or difficult diagnosis (e.g. seminal parameters that are close to the standard thresholds or UMI patients).

Poster 4

HDAC7, a novel biomarker and potential therapeutic target for infant pro-B-ALL with MLL-AF4 rearrangement

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Acute lymphoblastic leukemia (ALL) is the most common type of cancer in childhood. ALL stemming from developmentally-stalled B cell progenitors (pro-B lymphocytes) constitutes a specific subtype of B-ALL, commonly associated to MLL rearrangements. Pro-B-ALL carrying the t(4;11) encoding for MLL-AF4 oncogenic fusion shows a dismal clinical outcome (5-year survival, below 30%) with common therapy refractoriness and early relapses, especially in infants (younger than 12 months). Unfortunately, despite multiple worldwide efforts, no *bona fide* models recapitulating the t(4;11) MLL-AF4+ pro-B-ALL pathogenesis (latency and phenotype) exist, challenging our understanding of molecular mechanisms and biomarkers underlying the pathogenesis of this malignancy.

Previous research from our group has demonstrated that the histone deacetylase HDAC7 is a key transcriptional repressor during the early steps of B cell differentiation in mouse models. Moreover, we have also shown that HDAC7 is under-expressed in B-ALL cell lines and primary samples from adult patients with pro-B-ALL. Importantly, a forced ectopic expression of HDAC7 in B-ALL cell lines induces apoptosis, leads to the downregulation of c-Myc oncogene expression and inhibits their oncogenic potential *in vivo*, in xenograft models.

Here, we report that HDAC7 is significantly downregulated in MLL-AF4+ pro-B-ALL infants (n=14). Indeed, the ectopic overexpression of an MLL-AF4 construct critically downregulates HDAC7 levels, while it induces c-Myc expression. Remarkably, in a large cohort of MLL-AF4+ pro-B-ALL infants (n=40), lower levels of HDAC7 are associated with a shorter overall survival of these patients. In order to corroborate the potential anti-oncogenic effect of re-expressing HDAC7 in infant MLL-AF4+ pro-B-ALL cells, we have generated a stable pro-B-ALL SEM-K2 cell line expressing a Tet-On/Tight inducible double plasmid system that permits the activation of HDAC7 gene upon doxycycline treatment. Interestingly, both proliferation and cell viability were significantly compromised after 48-72 hours of doxycycline-mediated HDAC7 induction. Additionally, the activation of HDAC7 in SEM-K2 cells led to the repression of RUNX1 and FLT3 (both at mRNA and protein levels), suggesting that the absence of HDAC7 may contribute to the disease pathogenesis by allowing the expression of these key downstream targets of MLL-AF4, previously found involved in the evolution of the disease from a pre-leukemic clone into an overt leukemia.

This study represents an initial advance in deciphering the relevance of HDAC7 downregulation and the mechanisms underlying HDAC7 repression in driving pathogenesis of infant MLL-AF4+ pro-B-ALL. The identification of these mechanisms will eventually facilitate the development of novel therapeutic strategies aiming to treat this type of leukemia, which still remains as the deadliest among pediatric B-ALL.

Poster 5

SWI/SNF subunits Brg1 and Brm affect alternative splicing by regulating the recruitment of splicing associated factors.

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SWI/SNF ATP dependent chromatin remodelling complexes are involved in the regulation of mRNA processing, in particular on splicing and polyadenylation. Although some models have been proposed, little is known about the role of SWI/SNF complexes in alternative splicing. The aim of our study is to investigate the mechanism by which SWI/SNF complexes are involved in RNA processing. We identified a subset of genes which display a change in the exon inclusion/exclusion ratio upon expression of the core SWI/SNF ATPases Brg1-wt (23 exons) or Brm-wt (9 exons) in the SWI/SNF defective cell line C33A. Expressing the ATPase-mutated versions of Brg1 and Brm also affected the splicing outcome of genes, but little overlap between genes was observed. Several Brg1 and Brm binding partners were identified in an RNAse treated chromatin fraction by MASS spec, many of which were mRNA processing factors. Several of these factors, such as hnRNPL, hnRNPU, SYF1, SAM68 and DXH15 were recruited to affected exons upon Brg1 and Brm expression. Chromatin RIP showed that Brg1 and Brm changed the binding of the factors to RNA. Our results suggest that SWI/SNF core subunits favour the recruitment of several splicing associated factors to target exons in a gene specific way. We propose that SWI/SNF affects the interaction of factors with the growing RNA chain and that the effect is context specific.

Poster 6

HDAC11 deficiency induces a shift in skeletal muscle fiber type

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ABSTRACT

Histone deacetylases (HDACs) are essential epigenetic regulators of gene transcription. Histone deacetylase 11 (HDAC11) is the unique member of the class IV HDAC subfamily and its role in skeletal muscle physiology has never been addressed. In the present study, we examine skeletal muscle tissue in wild type (WT) and HDAC11 knockout mice ($HDAC11^{-/-}$). Loss of HDAC11 has no obvious impact on skeletal muscle histology neither in muscle growth. However, lack of HDAC11 promotes a fast-to-slow muscle fiber switch, increasing the number of oxidative myofibers. Transcriptomic analyses of WT and $HDAC11^{-/-}$ skeletal muscle show no major differences in the expression levels of genes encoding slow- and fast-type transcription factors, sarcomeric proteins and intracellular calcium channels. These results suggest that HDAC11 is not a major transcriptional repressor of slow-twitch genetic program. Interestingly, loss of HDAC11 increases mitochondrial DNA content in fast-twitch skeletal muscles, where HDAC11 is higher expressed compared to slow-twitch muscles. Moreover, we observe higher phospho-AMPK and phospho-ACC levels in HDAC11 deficient fast-twitch muscles, being both activities implicated in the regulation of fatty acid oxidation. Currently, we are analyzing the oxidative capacity of muscle tissue to address the metabolic reprogramming towards lipid metabolism. In summary, our results suggest that HDAC11 is a metabolic regulator in skeletal muscle and its lack promotes fatty acid metabolism.

Poster 7

Impact of PHF8 histone demethylase in astrocytes differentiation.

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PHD finger protein 8 is a histone demethylase specific for H4K20me1 and H3K9me2 histone marks and it plays a pivotal role in cell cycle progression, rDNA transcription and brain development. PHF8 mutations have been found in patients with X-linked intellectual disability and cleft lip/palate or autism and many of these mutations impair the histone demethylase catalytic activity. Considering the importance of histone methylation/demethylation during neural development and the lack of molecular data about PHF8 involvement in lineages specification, we investigated the role of PHF8 in glial lineage specification revealing an important contribution of PHF8 in astrocytes differentiation and function. Moreover, the above-mentioned phenotype is due to the lack of PHF8 HDM activity, as demonstrated by rescue experiments, and PHF8 catalytic activity is indeed affected in X-linked intellectual disabilities and autism spectrum disorders. Overall, we demonstrate that PHF8 is essential for proper astrocytes differentiation and that its depletion affects synapse formation and maturation.

Poster 8

Differential microRNAs expression profile in the hippocampus of senescent mice offspring induced by maternal resveratrol supplementation

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Nowadays, several studies demonstrate that epigenetic is an important process that regulates the progression of normal and pathological aging and neurodegenerative diseases such as Alzheimer's disease (AD). MicroRNAs (miRNAs), a small non-coding RNAs, are involved in gene expression regulation. These molecules promote the post-transcriptional silencing of their target mRNAs, and alterations in miRNA expression profiles have been associated with aging and cognitive and neurodegenerative disorders. Furthermore, environmental factors such as lifestyle, stress, exercise or diet can modulate epigenetic mechanisms, improving health phenotypes, which can be transmitted across generations. The Senescence-Accelerated Mouse Prone 8 (SAMP8) can be a useful tool for understanding the epigenetic processes during the pathological aging and their transmission across generations. In the present study, we investigated the effect on miRNAs expression profile induced by maternal resveratrol supplementation before pregnancy in the hippocampus of SAMP8 offspring. The offspring was generated from females SAMP8 fed with a resveratrol-enriched diet for two months before mating. We analyzed 22 different miRNAs that are known to be altered in SAMP8 and involved in neurological diseases. Compared with the SAMP8 Control, both F1 and F2 from resveratrol fed mother showed differentially expressed 9 miRNAs (*mmu-let-7e-5p*, *mmu-miR-128-3p, mmu-miR-140-5p, mmu-miR-148b-3p, mmu-miR-181a-5p, mmu-miR-29c-3p, mmu-miR-431-5p,* mmu-*miR-298-5p* and *mmu-miR-101b-3p)* at 6 months of age. Finally, we used bioinformatics approaches TargetScan, miRanda and PicTar software to predict target mRNAs and integrate with our miRNA data. Then, we explored the regulatory networks using GeneMania, an App from Cytoscape. These results indicated changes in miRNA expression profiles induced by resveratrol supplementation that may be of particular interest for understanding the mechanism of age-related cognitive decline, as well as helping to clarify a new neuroprotective mechanism of resveratrol.

ACKNOWLEDGEMENTS: This study was supported by Ministerio de Economíay Competitividad of Spain and FEDER (SAF2016-77703), PCIN-2015-229, and 2017SGR106 (AGAUR, Catalonia). Financial support was provided for V.I. (University of Barcelona, APIF_2017) and V.P.-Á. (University of Guadalajara, V/2014/2016).

Poster 9

H4K5 post-translational modifications dynamics on sperm chromatin remodeling

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During the last phase of the human spermatogenesis, the nucleohistone to nucleoprotamine (NH-NP) transition takes place, giving rise to a unique highly condensed chromatin structure essential for sperm functionality. However, this chromatin remodeling is not complete, and around 5-15% of the histones remain retained in mature human sperm. It is known that histones carry post-translational modifications (PTMs) that are essential for the regulation of sperm chromatin remodeling, such as the well-known lysine acetylation (Kac) and the recently discovered lysine butyrylation (Kbu). Interestingly, Bromodomain testis-specific protein (BRDT) binds to acetylated H4K5 (H4K5ac) and triggers the NH-NP transition, but when this residue is butyrylated (H4K5bu), histones escape the replacement, since BRDT is not capable of recognizing this acylation. Therefore, Kbu is directly competing with Kac. Unpublished immunohistochemistry results from our group in testicular biopsies of patients with normal spermatogenesis revealed high levels for both PTMs around the stages of elongated spermatids, which support the hypothesis of a dynamic H4K5bu-H4K5ac competing mechanism that controls the NH-NP transition. Of note, the presence of both H4K5ac and H4K5bu has been detected in mature sperm from normozoospermic semen samples in the present study, by Fluorescent Western Blotting. Further studies are required to unravel if this represents simply spermatogenic leftovers or, in contrast, might be acting as chromatin epigenetic marks with potential regulatory roles in early embryo development and zygote epigenetic inheritance. Future plans are focused on stablishing the levels of H4K5ac and H4K5bu in mature sperm from normozoospermic semen samples and in sperm with altered chromatin structure. Additionally, to deeply study the relevance of both PTMs beyond fertilization, ChIP-seq analysis will be carried out. Following this line, our goal is to decipher what is hidden behind this differential regulation of NH-NP replacement and its impact in sperm chromatin remodeling and early embryo development.

Funding: this work was supported by grants from Ministerio de Economía y Competitividad (PI16/00346 to R.O, FI17/00224 to A.I and CD17/00109 to J.C.) and FPU15/02306 Ministerio de Educación, Cultura y Deporte to F.B.

Poster 10

Targeting the oncogenic role of the chromatin remodeler BRG1 in Neuroblastoma

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Epigenetic therapies targeting regulators of gene expression are a promising strategy for those cancers with drug resistance and poor survival rates, as in the case of high-risk neuroblastoma (NB). BRG1 is the core subunit of the SWI/SNF complex, an ATP-dependent chromatin remodeling complex, and has been widely related to cancer. While in lung or ovarian cancers its expression lost and acts as a tumor suppressor gene, in some cancers like melanoma BRG1 is overexpressed and essential for survival and proliferation.

We recently found that high BRG1 levels correlate with poor prognosis in NB and chemoresistant NB cells depend on BRG1 expression for their growth and viability. Our hypothesis is that the differential role of BRG1 in cancer depends on the subunit composition of the BRG1-containing SWI/SNF complex, which dictates its genomic occupancy and its downstream transcriptional targets. The aims of this study are to find the factors that lead to an oncogenic activity of this protein in NB and to exploit this knowledge to develop a new therapeutic strategy to modulate the oncogenic function of BRG1.

To determine if there is any specific SWI/SNF subunit related to the oncogenic function of BRG1, we analyzed the BRG1 interactome comparing BRG1-dependent and independent NB cells by mass spectrometry. We also performed a whole transcriptome analysis of BRG1-dependent and independent NB cell lines after knockdown of BRG1 to describe the transcriptional programs regulated by this protein in oncogenic and non-oncogenic scenarios. Finally, we characterized the integrity and composition of the remaining SWI/SNF complexes after BRG1 knockdown in BRG1-dependent and independent cells.

These findings, together with further research, will make possible the understanding of the bivalent function of BRG1 in cancer and the detection of new therapeutic targets to inhibit the oncogenic function of the SWI/SNF complex in NB.

Poster 11

3D genome structure reconstruction from sparse 3C-based datasets

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3C technologies measure the interactions frequency between chromatin regions within the three-dimensional (3D) nuclear space in a population of cells. One of such technologies is Promoter Capture HiC (pcHiC) that measures interactions involving promoters, resulting in sparse interaction matrices that are rich in long-range enhancer-promoter interactions. Here, we introduce a new method to generate chromatin 3D models from pcHiC data combining a new normalisation protocol to handle sparse data matrices with TADdyn polymer-physics based restrain modelling. We use this method to investigate the 3D organisation of loci containing promoters related to diabetes onset. We unveil that enhancers coming from linearly distal genomic regions physically co-localise around the promoter, constituting 3D enhancer hubs in Pancreatic Islet (PI) cells.

Poster 12

Dynamic regulation of chromatin through 3D epigenetic clusters

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During embryonic development, proper orchestration of gene expression programs is accompanied by precise epigenetic and topological rearrangements of chromatin, tightly regulated in both space and time. Unfortunately, the link between gene expression, epigenetic modifications and genome architecture is still poorly understood.
To get a unified vision of epigenetic and topological features of the genome, we aim to characterize *three-dimensional (3D) epigenetic clusters*. These are sets of chromatin elements that recurrently colocalize in the nucleus with possible implications in gene transcription. Chromatin elements include histone modifications, DNA-binding proteins, and functional elements such as promoters and enhancers.
To address this challenge, we have developed and applied *3DEpINT*, a new computational method to integrate chromatin structure data from Hi-C interaction matrices and epigenetic data from ChIP-seq profiles. Explicitly, we define an epigenetic coefficient for each pair of chromatin loci, weighted on their three-dimensional physical interaction in the cell nucleus. Thanks to this, we are able to automatically detect chromatin long-range physical interactions concomitant with enrichment for epigenetic marks, which correspond to 3D epigenetic clusters.
Investigating their dynamic changes during mouse embryonic stem cell differentiation in a quantitative and genome-wide manner will help deciphering the complex relationship between genome function, its 3D architecture and the epigenome.

Poster 13

BET protein as a novel target to reduce inflammation and enhance functional recovery after spinal cord injury

Judith Sánchez-Ventura, Xavier Navarro, Clara Penas

Spinal cord injury (SCI) usually causes a devastating lifelong disability for patients. After a traumatic lesion, disruption of the blood-spinal cord barrier induces the infiltration of macrophages into the lesion site, and the activation of resident glial cells, which release cytokines and chemokines. These events result in a persistent inflammation, which has both detrimental and beneficial effects, but eventually limits functional recovery and contributes to the appearance of neuropathic pain. Bromodomain and extra-terminal domain (BET) proteins are epigenetic readers that regulate the expression of inflammatory genes by interacting with acetylated lysine residues. While BET inhibitors are a promising therapeutic strategy for cancer, little is known about their implication after SCI. Thus, the current study was aimed to investigate the anti-inflammatory role of BET inhibitors in this pathologic condition. We found that the BET inhibitor JQ1 reduced the levels of pro-inflammatory mediators, and increased the expression of anti-inflammatory cytokines. Treatment with JQ1 also decreased reactivity of microglia/macrophages, enhanced neuroprotection and functional recovery, and acutely reduced neuropathic pain after SCI. These novel results demonstrate for the first time that targeting BET proteins is an encouraging approach for SCI repair and a potential strategy to treat other inflammatory pathologies.

Poster 14

Identification of gonadal miRNAs in zebrafish exposed to high temperature during early stages of development

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Keywords: epigenetic, high temperature, biomarkers

MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression in a wide variety of physiological processes. In some fish species (e.g., rainbow trout and medaka), it has been shown that miRNAs play regulatory functions in the reproductive system and that some of them are specific to the fish gonads. However, how miRNAs change their gonadal dynamics in fish subjected to environmental factors (i.e., high-temperature) during early stages of life, has not been studied yet. In order to understand the role of the epigenetic regulation mediated by miRNAs in the gonads, we exposed zebrafish (*Danio rerio*) to elevated temperatures during early development (18-32 days post fertilization, dpf), a treatment that is known to result in male-skewed sex ratios. Once the fish reached adulthood (90 dpf), 8 ovaries and 8 testes were dissected and kept at -80ºC. MiRNAs were isolated and specific small RNA libraries were prepared and sequenced by Illumina technology (50 bp 1x50, v4, HiSeq). About 8 million reads were obtained from gonadal samples. Sequencing results were analyzed by miRDeep2 software that allowed, with high accuracy, to trim the sequencing data, to detect known miRNA from several databases and to identify novel miRNAs. Analysis of the expression levels of the miRNA identified a total of 25 and 1 unique miRNAs in ovaries and testes, respectively. After retrieving UTR regions, we analyzed these 26 identified miRNA by Miranda software and after filtering, we obtained almost 400 potential RNA targets. Currently, validation of the differentially expressed miRNAs by qPCR analysis is being performed. This study will provide a catalogue of both sex-specific and thermosensitive miRNAs in the zebrafish gonads that might be used as potential molecular biomarkers of the effect of temperature during sex differentiation.

Poster 15

JMJD3-mediated chromatin re-organization controls transcription upon TGFβ signaling

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JMJD3 is a histone demethylase which catalyzes the removal of the repressive marks H3K27me2/3. During neural development it participates in the commitment and differentiation of neural stem cells to the neuronal lineage. The molecular mechanisms underlying this regulation are starting to be uncovered. In this work, we have used neural stem cells as a model to unravel JMJD3 role during early neurogenesis. We have shown that in response to TGFβ, JMJD3 regulates specific gene expression programs by promoting neuronal enhancers' activation. To do that, it participates in the establishment of enhancer-promoter and enhancer-enhancer contacts in a TGFβ-dependent manner, which in turn leads to enhancer and gene expression activation. Finally, our data suggest that JMJD3 fine-tunes chromatin conformation by promoting a liquid-liquid de-mixing process, which contributes to the formation of a functional super-enhancer where co-activators and transcription factors accumulate to prompt gene expression.

Poster 16

Does chromatin metabolism drive breast cancer evolution?

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We want to study the role of cancer metabolism in triple negative breast cancer with the final aim to identify a targeted therapy for this unmet medical need. The treatment of breast cancer is still a worldwide health challenge, due to the high intrinsic heterogeneity of the disease. There are three fundamental types of breast cancer, which are characterized by the expression ER, PrR and/or HER2 or by the absence of all those three receptors (triple negative breast cancer). The triple negative is the most aggressive one because no targeted therapy has been found until now, fact that limits the treatment to non selective and aggressive chemotherapeutic agents, and strongly reduces the probability of patient survival.

The focus of our team is to study the role of cancer metabolism in epigenetic and transcriptional regulation. It has become evident during the last years that certain metabolic enzymes localizes on chromatin despite their canonical cytoplasmic localization and function. We aim to study the role of metabolic enzymes specifically recruited on chromatin in triple negative breast cancer, to understand their contribution to tumor progression. For this, we started setting up a protocol for chromatin purification MS-coupled, which will allow us to investigate the chromatome of selected breast cancer cell lines recapitulating the different breast cancer phenotypes. By comparing the chromatome of those cell lines, we will select enzymes specifically recruited to chromatin in triple negative breast cancer and investigate what is their function there. The identification of a metabolic enzyme whose chromatin localization is selectively required in triple negative breast cancer could open up new possibilities for the successful treatment of this aggressive tumor.

Poster 17

Cis-Regulatory Element (CRE) dynamics during planarian posterior regeneration

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Organizers or signaling centers are a group of cells with the ability to specify the fate of adjacent cells, allowing a patterned growth. The activity of organizers relies in the secretion of signaling molecules, or morphogens, which are received and interpreted by the surrounding cells. Organizers are well studied in embryonic models. However, their function is also required in adult tissues, for instance for patterning regenerating organs. In planarians, flatworms that are able to regenerate any missing body part, the anterior and the posterior tips behave as organizers. The posterior organizer is defined by the expression of *wnt1*, and its inhibition leads to tailless or two-headed planarians. The anterior organizer is defined by the expression of *notum*, a secreted inhibitor of Wnt, and its inhibition leads to headless or two-tailed planarians. It is known that until 24 hours of regeneration both *wnt1* and *notum* are expressed in any wound. At around 36 hours their expression is restricted to the posterior or the anterior tip, respectively, forming the organizers. To further understand the molecular interactions that finally allow the formation of the specific organizer in each tip we are performing ATAC-seq and ChIP-seq analysis of anterior and posterior regenerating blastemas of wild-type, *wnt1* RNAi and *notum* RNAi animals. We have characterized specific Cis-Regulatory Elements (CREs) of anterior and posterior regeneration and compared them with active CREs of *wnt1* and *notum* RNAi animals. We have found that already at 12 hours of regeneration the accessible CREs in anterior blastemas of *notum* RNAi animals are almost identical to the ones found in wild-type posterior blastemas, indicating that specific anterior or posterior chromatin changes induced by amputation occur much earlier than the formation of the organizers. We have also identified transcription factors specifically related to posterior CREs and thus essential for posterior identity specification.

Poster 18

The DNA methylation of the Kallikrein locus as a pancancer biomarker

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Aberrant DNA methylation is a hallmark of cancer and has been used as a diagnostic and prognostic biomarker in several cancers. The human tissue Kallikrein (KLK) gene family is constituted by 15 members located in a 265 kb cluster on chromosome 19. KLKs are secreted serine proteases implicated in a vast range of physiological processes and pathologies. They are highly dysregulated in cancer, where they have a dual role being oncogenic or tumor-suppressor, and constitute an important source of tumor biomarkers. It has been reported that some KLKs are under the regulatory control of DNA methylation. Using TCGA data from 9,865 samples covering 32 cancer types, we observed differences between normal and cancer tissue in the KLK locus methylation. Interestingly, while there is a general hypomethylation of the cluster, some specific cancers show a global hypermethylation. Importantly, this difference is greater in recurrent and metastatic samples. In addition, the aberrant methylation of the KLK locus in primary tumors is associated with mortality and recurrence in a pancancer analysis. Finally, the expression of some kallikreins and the methylation of the KLK locus correlate in some cancers pointing to a functional role of the epigenetic status of the locus. In conclusion, we propose the DNA methylation status of the KLK locus as a novel pancancer diagnostic and prognostic biomarker.

Poster 19

Characterization of human sperm protamine proteoforms through a combination of top-down and bottom-up mass spectrometry approaches

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Abstract:
The protamine 1 (P1) and protamine 2 (P2) family members are the most abundant basic proteins in human spermatozoa and pack 85-95% of the paternal genome. P1 is synthesized as a mature form, whereas P2 components arise from the proteolysis of the precursor (pre-P2). Due to the particular physical-chemical properties of protamines, its identification by standardized bottom-up MS strategies based on trypsin-digestion is not straightforward. Furthermore, this approach does not allow to directly identify proteoforms -or precise molecular forms of proteins-, which are critical to understanding their function. Therefore, the aim of this study was to identify P1 and P2 family members and their post-translational modifications (PTMs) pattern using two complementary approaches: (i) a top-down MS approach with novel proteomic workflows and data analysis pipelines; and (ii) a bottom-up MS approach based on proteinase K-digestion, rather than trypsin-digestion. The top-down MS approach allowed identifying intact naïve P1, pre-P2 and P2 mature forms and their phosphorylation patterns. Additionally, uncharacterized truncated proteoforms were identified in both P1 and pre-P2 (spanning residues 8-51 and 8-102, respectively), suggesting its possible generation by proteolysis as occurs in P2 mature forms. Surprisingly, a +61 Da modification was found in different proteoforms, possibly corresponding to Zn^{2+} ion, supporting the hypothesis that Zn^{2+} could help to temporarily stabilize sperm chromatin until fertilization. From a different perspective, the bottom-up MS approach results permitted finding peptides from the pre-P2 with different phosphorylated residues and confirmed the existence of a new P2 splice variant, the isoform 2 of P2, with 2 unique peptides, one of them phosphorylated, validated also at the RNA level in an independent sample. These results open a window to study the role of protamine PTMs and its potential function as epigenetic signatures potentially involved in the replacement of paternal protamines by maternal histones after fertilization.

Funded by: *Ministerio de Economía y Competitividad* (PI13/00699, and PI16/00346), EUGIN-UB-2014 to RO. M.J. is granted by Government of Catalonia (Generalitat de Catalunya, pla estratègic de recerca i innovació en salut, PERIS 2016-2020, SLT002/16/00337). J.C. is supported by the Sara Borrell Postdoctoral Fellowship from the Spanish Ministry of Economy and Competitiveness (Ministerio de Economía y Competitividad, Acción Estratégica en Salud, CD17/00109).